Cell count of *iLite*® Assay Ready Cells

For research and professional use only. Not for use in diagnostic procedures.
This technical note is written to illuminate some of the principle issues for iLite® Cell Based Technology. Each laboratory must set up their own method and perform relevant validations.

Background

The certificate of analysis for each batch of *iLite*® Assay Ready Cells state the nominal cell concentration for the product. As part of the quality control (QC) procedure for the products, the cell batch go through a QC-test assuring the batch fulfilling the QC criteria for functionality, viability and cell concentration. The QC procedure is performed on multiple vials since cell counting and viability determination is associated with considerable uncertainty.

Cell Counting

There are some principle issues connected to cell counting and inherent uncertainty attached that may in the end have large impact in conclusions drawn from a cell count and viability assay. These are 1) Sampling 2) Dilution and 3) Cell Size.

1. Sampling

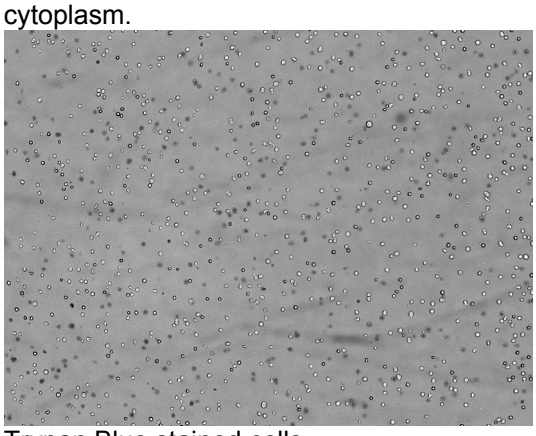
Cell counting and viability determination is performed on a very small sample of the total cell solution in question. Usually a sample of ex. 10 µl diluted and stained cell solution is added a cell counting chamber, but only 0.4 µl of this sample is actually counted since a typical counting chamber space is only 4 × 1 mm^2 with a depth of 0.1 mm.

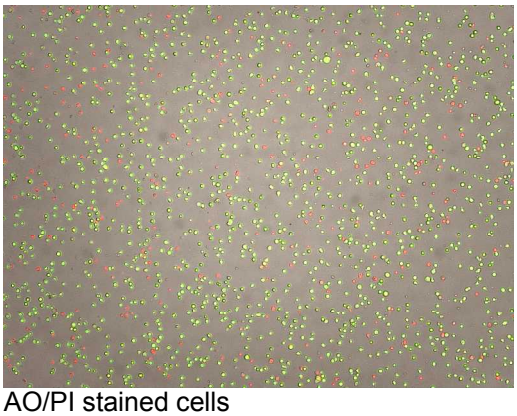
2. Dilution

When diluted as recommended in the application notes for the *iLite*® Assay Ready Cells, the cell concentration of the cell solutions may vary from 0.5 x 10^6 cells/mL to 5.0 x 10^6 cells/mL and the dilution factor performed vary from 3.2 to 24.
Cell counting is usually performed in the concentration range from 0.25 x 10^6 cells/mL to 2.5 x 10^6 cells/mL. Additional dilution is therefore applied to stain the cells prior to the cell count/viability test. Combined, these factors mean that cell counting numbers around 100 is not unusual. The implication of that is that cell concentration and viability evaluation are based on very few observations and is therefore associated with some degree of uncertainty.

3. Cell size

The cell size matters when the principle of dye exclusion is used for viability evaluation. Trypan Blue Staining Assay is one of the most common methods for measuring cell viability. Trypan blue is an azo dye that is cell membrane impermeable and therefore only enters cells with compromised membranes (unviable cells). A viable cell will have a clear cytoplasm whereas a nonviable cell will have a blue cytoplasm.


Trypan Blue stained cells


AO/PI stained cells

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Cell lines with particularly small cell size, like *iLite*® Assay Ready Cells based upon cell line DT-40, may become problematically in the Trypan Blue Staining Assay, since it might be hard to distinguish cells with clear from blue cytoplasm. Therefore, the use of fluorescent dye like acridine orange (AO) and propidium iodide (PI) typically gives more consistent results counting small sized cells. AO and PI are both nuclear staining (nucleic acid binding) dyes. AO is permeable to both live and dead cells and stains therefore all nucleated cells to generate green fluorescence while PI enters dead cells with compromised membranes and stains all dead nucleated cells to generate red fluorescence.

General recommendation for cell counting related to *iLite*® Assay Ready Cells

iLite® Assay Ready Cells can be examined using the standard techniques (i.e trypan blue staining) for cell counting and viability testing, but the result should only be considered as **indicative** and should not be used for adjustment of cell concentration prior to an assay. If normalization for cell number and viability per well is wanted, this can be achieved with less uncertainty by using the normalization Renilla gene expression present in most *iLite*® cell lines.
It is recommended to use fluorescent dye (i.e AO/PI staining) if cell counting and viability testing is performed of *iLite*® Assay Ready Cells based upon host cell line DT-40, since these cells are particularly small. Please refer to each Product Specification regarding host cell line of the *iLite*® Assay Ready Cell.

Troubleshooting and FAQ

Please consult the Svar Life Science website www.svarlifescience.com